

Effect of Three Different Remineralizing Agents on White Spot Lesions; An In Vitro Comparative Study

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Abstract

Background and Aim: White spot lesions (WSLs) are common occurrence following orthodontic treatment, this study sought to compare the remineralizing potential of fluoride, MI Paste Plus (CPP-ACP-F) and Remin Pro for treatment of WSLs.

Materials and Methods: This in vitro study was conducted on 56 sound human premolars that extracted for orthodontic purposes. Teeth were cleaned and their roots were cut, then the crowns were mounted in acrylic resin. Teeth surfaces were coated with nail varnish except for a 3×4mm window on the buccal surface. In order to create WSLs, all samples were immersed in demineralizing solution for 10 days. After surface hardness was measured using a Vickers microhardness tester, teeth were randomly divided into four groups. In control group (group 1) no remineralizing agent was used while, groups 2 to 4, were treated with 0.05% sodium fluoride (NaF), MI Paste Plus, and Remin Pro paste respectively, for five minutes every 12 hours. After each treatment, specimens were immersed in freshly prepared artificial saliva. At the end of 28 days, surface microhardness was measured again and changes were statistically analyzed using analysis of covariance (ANCOVA).

Results: The mean changes in surface microhardness of MI Paste Plus, NaF and Remin Pro groups was significantly higher than control group ($P \leq 0.001$, $P \leq 0.001$ and $P = 0.046$ respectively).

Conclusion: MI Paste Plus, 0.05% NaF mouthwash, and to a lesser extent Remin Pro were efficient for treatment of WSLs comparing to the artificial saliva.

Key Words: Dental Caries, Sodium fluoride, Mouthwashes, Therapeutics

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Introduction

Enamel demineralization following orthodontic treatment is a common concern for orthodontists, patients, and their parents [1,2]. Increased prevalence of these lesions following fixed orthodontic treatment is mostly due to the escalation in plaque accumulation on tooth surfaces because of irregular surfaces of orthodontic

brackets, bands, wires, and other appliances. Moreover, placement of appliances on dental surfaces complicates oral hygiene and limits the self-cleaning capacity of teeth with the saliva flow and movements of oral muscles [1]. According to the literature, the prevalence of WSLs following orthodontic treatment is varied between 2 to 96% [1,2], and they mainly happen on cervical and

middle thirds of the buccal surface of first molars, lateral incisors and canine teeth [2]. Despite extensive studies on prevention of WSLs, they remain a problem following orthodontic treatment [3]. In WSLs, the light scattering pattern on demineralized enamel creates a chalky white appearance, which is not aesthetically pleasant [2]. Approximately half of the WSLs heal spontaneously after orthodontic treatment is terminated and proper oral hygiene is established via natural remineralization function of ions present in the saliva. However, coloring agents in foods and beverages may penetrate into the lesion and create an unaesthetic appearance. In deep lesions, remineralization does not occur if no therapeutic intervention is carried out [4].

Several methods have been suggested for treatment of these lesions. The first preventive measure and treatment are proper oral hygiene in orthodontic patients. Other therapeutic approach includes the use of fluoride-containing products such as fluoridated toothpastes, mouthwashes containing fluoride, fluoride gels, varnishes, and cements, which release fluoride. Application of CPP-ACP paste (casein phosphopeptides and amorphous calcium phosphate), antiseptics, laser irradiation, bleaching techniques, resin infiltration method, and invasive techniques such as micro-abrasion and composite restorations are other available treatments [4,5].

Fluoride ions decrease enamel solubility and enhance its acid resistance when incorporated into the mineral tooth structure [1]. However, concentrated fluoride is not recommended for the treatment of WSLs on the labial surface of anterior teeth since the excess fluoride absorption could cause some degree of enamel fluorosis which results in the darker and dull tooth color [4].

The CPP-ACP is a calcium phosphate-based cement, which enhances the process of enamel remineralization by increasing the available calcium and phosphorous ions. Furthermore, the remineralized surface following the use of CPP-ACP is esthetically acceptable [4]. Moreover, previous studies showed that use of fluoride along with CPP-ACP increased the remineralization efficacy [4,5].

Remin Pro is a recently introduced remineralizing agent for prevention and treatment of enamel

demineralization [6]. It is a water-base cream, which contains hydroxyapatite and fluoride [7]. It has been claimed that due to the absence of bovine proteins, which is existed in CPP-ACP, Remin Pro is preferred in patients with the bovine proteins allergy [6].

Search of the literature yielded no previous study comparing the efficacy of Remin Pro, CPP-ACP-F and fluoride. Thus, this in vitro study aimed to assess and compare the efficacy of Remin Pro, CPP-ACP-F and NaF in treatment of WSLs.

Materials and Methods

This in vitro experimental study was conducted on 56 sound maxillary and mandibular premolars which were extracted for orthodontic purposes. Teeth had no discoloration, caries, restorations, cracks, and/or fractures. After teeth were collected, they were cleaned with a low-speed handpiece, prophylaxis brush, and pumice paste, and disinfected with 0.1% thymol solution for 48 h, then stored in saline. Subsequently, the roots were cut by high-speed handpiece and long fissure bur and the crowns were coated with nail varnish except for a 3×4mm window at the center of the buccal surface followed by mounting in autopolymerizing acrylic resin (Acropars, Tehran, Iran). The demineralizing agent was prepared in the chemical laboratory of Jahad Daneshgahi, Tehran University of Medical Sciences with the pH of 4.5. [2.2mM CaCl₂ (Merck, USA), 2.2 mM NaH₂PO₄ (Merck, USA), 50mM acetic acid (Merck, USA), 100 mMNaCl (Merck, USA), 1ppm NaF (Merck, USA), and 0.02% NaN₃ (Merck, USA)].

In order to create WSLs on the surface of specimens, each sample was immersed in 10ml of the demineralizing solution at room temperature (approximate 25°C) for 10 days. After 10 days and verification of WSL appearance, surface microhardness (SMH) of each sample was measured using a microhardness tester (V-Test, Baresiss, Germany) in three points with a Vickers diamond indenter under a load of 200N for 10 seconds. Afterward, specimens were randomly divided into four groups of 14teeth. Each group was immersed and kept in artificial saliva solution [0.04wt% KCl (Merck, USA), 0.04wt% NaCl (Merck, USA), 0.09wt% CaCl₂. 2H₂O (Merck, USA), 0.069wt% NaH₂PO₄. 2H₂ (Merck, USA),

0.008wt% $\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$ (Merck, USA), 0.05wt% urea (Merck, USA), 0.01wt% ascorbic acid (Merck, USA), 10ml water, 0.15 mg methyl-p-hydroxybenzoate (Merck, USA), 7wt% PEG 6000 (Merck, USA), 0.1wt% glucose (Merck, USA), and the pH was set at 6.9]. for 28 days and subjected to the different treatment protocol. Every specimen in group 1 (control group) was immersed in 10ml artificial saliva and no other treatment were applied and artificial saliva was refreshed twice a day.

In group 2 samples were immersed in 10 ml of 0.05% NaF mouthwash (Colgate® Neutra Fluor 220 Alcohol Free Mouthwash, USA) for five minutes every 12 hours (twice a day), then without rinsing, samples were immersed in a fresh artificial saliva solution.

Specimens in group 3 were treated with MI Paste Plus (GC America, Alsip, Ill) every 12 hours for five minutes then paste was wiped without rinsing, followed by immersing specimens in a freshly prepared artificial saliva solution.

Specimens in group 4 were prepared using the same procedure as described for group 3 except Remin Pro was applied to tooth surfaces.

At the end of the treatment protocol, microhardness of specimens was measured and changes in surface microhardness of samples were calculated. Collected data were statistically analyzed using analysis of covariance (ANCOVA).

Results

Statistical analysis revealed microhardness of all groups increased after 28 days of treatment protocol compared to the baseline measured after demineralization. The maximum increase in microhardness was observed in group 3 which treated with MI Paste Plus, this was followed in descending order by group 2 (NaF), group 4 (Remin Pro), and control group (Table 1).

Analysis of covariance (ANCOVA) evaluated the effect of the remineralizing agent on the final SMH while considering the effect of the initial SMH (SMH after demineralization). This analysis showed that initial SMH had a significant effect on the final SMH ($P \leq 0.001$) however, the interaction between initial SMH and the remineralizing agent was not significant ($P=0.106$). Since no interaction between SMH and the remineralizing agent was

found, ANCOVA revealed the significant effect of remineralizing agents compared to the control group. As it has been mentioned before, the final mean surface microhardness differences were statistically significant between the control group and MI Paste Plus ($P \leq 0.001$), NaF ($P \leq 0.001$), and Remin Pro ($P=0.046$).

Discussion

In a systematic review by Chon et al. [4] on the effect of remineralizing agents on WSLs after orthodontic treatment, they have suggested that NaF, CPP-ACP, and MI Paste Plus are effective materials for treatment of enamel lesions. However, no consensus has been reached about the most suitable and reliable technique for treatment of WSLs. Thus, this in vitro study aimed to compare the effects of NaF, MI Paste Plus, and Remin Pro on WSLs and to find the most effective, non-restorative treatment course for these lesions in orthodontic patients. For this purpose, we compared Remin Pro, a water-based paste containing hydroxyapatite and fluoride, with efficient and commonly used products such as NaF mouthwash and MI Paste Plus. The manufacturer claims that Remin Pro is an effective material for prevention and treatment of enamel lesions in orthodontic patients.

In the current study, a demineralizing solution with a pH of 4.5 was used to induce WSLs [8]. The main composition of demineralizing agents often includes calcium, phosphorous and acetic acid or lactic acid, which is determined based on the desired pH and duration of exposure for the creation of lesions. Based on the previous studies, the pH of demineralizing solutions varied from 3.5 to 5, also, duration of immersion in the solution ranged from two hours to 21 days [9-11]. In the present study, in order to create WSLs, the samples were immersed in a demineralizing solution with a pH of 4.5 for 10 days.

Different methods have been used to assess the degree of remineralization; for instance, Sudjalim et al. [1] used quantitative light-induced fluorescence (QLF) to assess the fluorescence change (ΔF) between baseline and after treatments. Mohan et al. [12] evaluated the surface morphology of the test samples by scanning electron microscopy. Digital imaging has also

Table 1. Means \pm standard deviations of Surface Micro Hardness (SMH) before and after remineralization

Groups	SMH after demineralization	SMH after remineralization	Change of SMH
Control	41.40 \pm 32.92	67.2 \pm 40.76	25.80 \pm 13.87
0.05% Sodium fluoride	53.57 \pm 32.34	111.56 \pm 48.58	57.99 \pm 25.93
MI Paste	65.24 \pm 30.33	126.47 \pm 43.34	61.23 \pm 23.63
Remin Pro	62.14 \pm 76.09	107.87 \pm 84.50	45.73 \pm 22.83

been used to compare the aesthetic change of white spot lesions which treated with 3 different products [2]. Another method which has been employed for assessment of the mineral content of samples is DIAGNOdent [4]. Behroozibakhsh et al. [8] used more precise methods including micro-computed tomography to assess the mineral content of samples. One of the most common and reliable methods for this purpose is the assessment of change in surface microhardness using Vickers microhardness tester [3-8]. In the present study, microhardness values were measured after demineralization and remineralization of specimens. Statistical analysis showed after treatment, the maximum increasing microhardness occurred in group 3 that samples were treated with MI Paste Plus. Similar results were obtained by Shetty et al, [13]; in their study, the application of MI Paste Plus was more effective than CPP-ACP and NaF for the treatment of enamel lesions following orthodontic treatment. Ballard et al. [2] compared 3 commercially available materials (MI Paste Plus, a toothpaste containing Novamin and 5000 Prevident) and they realized that none of these products had any superiority over the others. However, in our study, MI Paste Plus and NaF were significantly superior to the control group. The difference in the results of two studies may be due to the use of a different method of evaluation for determining the effect of remineralization materials. Also, it could be attributed to the use of fluoride toothpaste in their study while we used fluoride mouthwash [2].

In our study, the second highest change in surface microhardness was recorded in NaF group, which was in agreement with the results of a systematic review by Bergstrand and Twetman [3] on methods of prevention and treatment of WSLs in 2011. They have provided evidence supporting the optimal efficacy of fluoride products for

prevention of WSLs. Lata et al. [14] compared the remineralization potential of NaF and CPP-ACP; they reported that the remineralization potential of NaF was higher than CPP-ACP. Also, the remineralization potential of fluoride alone was higher than that of CPP-ACP-F. Such a difference in the results might be due to the different methods of application of materials. In a study by Vyavhare et al, [15] the remineralization potential of fluoride, nano-hydroxyapatite, and CPP-ACP has been assessed and they identified the higher remineralization potential of fluoride. They also stated that CPP-ACP cannot serve as an alternative to fluoride.

Most previous studies revealed that NaF, in different concentrations and forms such as mouthwash and varnish, significantly enhanced the remineralization of WSLs [4]. More recent studies on the efficacy of CPP-ACP showed approximately similar effects of NaF [8,14,15]. However, it has been shown that combined application of MI Paste Plus which contains CPP-ACP, and fluoride is more effective in remineralization of enamel lesions compared to NaF and CPP-ACP alone [13]. In the current study, results of ANCOVA revealed a significant difference in microhardness of groups treated with MI Paste Plus, NaF, and Remin Pro with the control group.

The previous study on the remineralizing efficacy of Remin Pro has assessed the changes in mineral content of the enamel surface subjected to Remin Pro, Propolis, and acidulated phosphate fluoride in conjunction with CO₂ laser irradiation [12]. The results revealed that samples treated with acidulated phosphate fluoride along with laser irradiation had the highest mineral content, which was in agreement with our results. Several studies on Remin Pro have evaluated its effect on bleached teeth; for instance, Heshmat et al. [16] in 2016

evaluated and compared the effect of Remin Pro, MI Paste Plus, and natural saliva on the microhardness of bleached teeth. For this purpose, the microhardness of teeth was measured followed by the bleaching process with 35% hydrogen peroxide. Once more microhardness was measured after bleaching. The teeth were then treated with Remin Pro, MI Paste Plus, and natural saliva for 15 days and their microhardness was measured again for the third time. They reported that surface microhardness of specimens significantly decreased following bleaching; however, the use of Remin Pro, MI Paste Plus and natural saliva for 15 days significantly enhanced surface microhardness. In their study, Remin Pro, MI Paste Plus, and saliva all showed almost similar efficacy in increasing the surface microhardness of bleached enamel [16].

Although the current study yielded almost similar results and the final SMH in Remin Pro group was significantly increased compared to the control group, the standard deviation was relatively high which means the remineralizing effect of Remin Pro was not uniform.

Conclusion

Within the limitations of this in vitro study, the results showed that MI Paste Plus and to a lesser degree 0.05% NaF are effective for treatment of WSLs. Although Remin Pro had an efficacy superior to the artificial saliva, its remineralizing effect seems to be inconsistent.

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Soodeh Tahmasbi, Mahya Adhami, Azam Valian, Roya Hamedei declare that they had no conflict of interest.

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